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Affinity purification of proteins using expanded beds

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ABSTRACT

The use of expanded beds of affinity adsorbents for the purification of proteins from feedstocks containing whole or broken cells is described. It is demonstrated that such feedstocks can be applied to the bed without prior removal of particulate material by centrifugation or filtration thus showing considerable potential for this approach in simplifying downstream processing flow-sheets. A stable, expanded bed can be obtained using simple equipment adapted from that used for conventional packed bed adsorption and chromatography processes. Circulation and mixing of the adsorbent particles is minimal and liquid flow through the expanded bed shows characteristics similar to those of plug flow. Frontal analysis performed with the highly selective affinity system involving the adsorption of human polyclonal immunoglobulin G onto Protein A Sepharose Fast Flow indicate that the adsorption performance of the expanded bed is similar to that achieved when the same amount of adsorbent is used in a packed configuration at the same volumetric flow-rate. The adsorption performance of the expanded bed was not diminished when adsorption was carried out in the presence of intact yeast cells. Batch adsorption experiments also indicated that the adsorption characteristics of the affinity system were not greatly altered in the presence of cells in contrast to results from a less selective ion-exchange system. An expanded bed of Cibacron Blue Sepharose Fast Flow was used to purify phosphofructokinase from feedstock of disrupted yeast prepared by high pressure homogenisation without the need for prior removal of particulate material. The potential for the use of expanded beds in large scale purification systems is discussed.

INTRODUCTION

Many downstream processing flow-sheets involve liquids that contain particulates. Such liquids may be fermentation broths containing cells or preparations of broken and ruptured cells containing cell debris. Adsorption techniques are popular methods for the recovery of a wide range of biomolecules from such sources. However, application of particulate-containing feed stocks to conventional packed beds of adsorbent beads results in severe operational problems as a result of the trapping of particulate matter in the voids of the bed (Fig. 1a). These problems may be manifested by an increase in the pressure drop across the bed and the formation of a plug of trapped solids at the inlet of the bed. Although batch adsorption procedures carried out in stirred tanks can be used as an alternative approach, the adsorption characteristics are likely to be inferior to the use of a packed bed procedure.

An alternative solution may be the use of liquid fluidised or expanded beds. When a bed of adsorbent beads is expanded in an unconstrained configuration by up-flow of liquid, particulate material is able to pass through the bed without becoming trapped (Fig. 1b). When a critical minimum liquid velocity is exceeded the bed starts to expand and gaps occur between the adsorbent beads and these gaps widen as the liquid velocity increases, *i.e.*, the external voidage of the bed increases. At this stage particles can pass freely through the bed, but if the velocity of flow is increased further, such that it exceeds the terminal velocity of the adsorbent beads in the irrigating liquid, an unsatisfactory situation is reached where the adsorbent beads themselves are carried out of the bed.

The ability to be able to apply particulate-containing feed-stocks directly to adsorbent systems may eliminate the need for prior removal of cells and/or cell debris by centrifugation or filtration. At the end of the adsorption phase, remaining particulates in the voids of the bed can be washed away while the bed remains in an expanded configuration. Subsequently, adsorbed species can be eluted from

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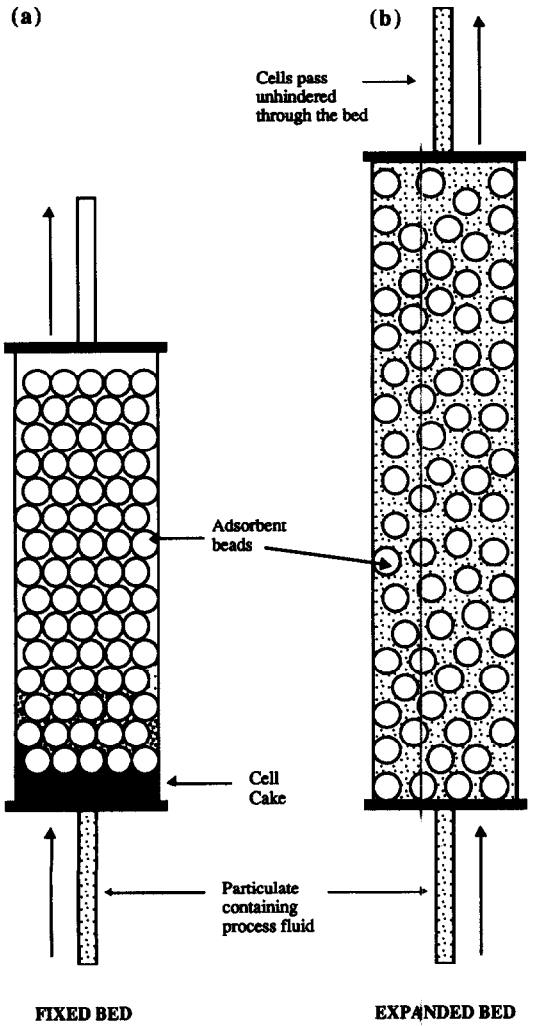

Fig. 1. Principle of the use of expanded beds for the adsorption of proteins in the presence of particulates. This schematic drawing demonstrates the trapping of particulates within the voids of a packed bed (a) and the avoidance of such problems if an expanded bed is used instead (b).

the bed by operation in either an expanded or packed configuration. Such use of expanded beds would greatly simplify the down-stream processing flow-sheets for a number of biomolecules with concomitant savings in equipment and operating costs. Fluidized beds have been used previously for the industrial scale recovery of some antibiotics including batch processing techniques for streptomycin [1] and semi-continuous systems for novobiocin [2]. However, more recently, considerable interest has again been shown in the use of expanded beds of adsorbent for the direct extraction of a wider range of biomolecules, including proteins, from whole fermentation broths or preparations of broken cells [3–13].

The traditional view of a bed of solid particles fluidised by the upward flow of a liquid is of a well-mixed system involving considerable circulation of the solids with associated mixing of the liquid [14]. Such conditions differ greatly from those obtained in a well-packed bed where the adsorbent beads are stationary and liquid flow approximates plug flow and would therefore be expected to show an inferior adsorption performance compared to the packed bed situation. Certain solutions have been proposed to minimise the circulation of particles in liquid fluidised beds for biomolecule recovery including the use of magnetically stabilised beds [15] or the division of the bed into sections achieved by the insertion of baffles [6,7]. In this paper, we shall review our work which demonstrates that a stable expanded bed of adsorbent beads can be established without the need to resort to such techniques. In addition, it will be shown that the adsorption performance of the expanded bed is very similar to the situation when the same amount of adsorbent is used at the same linear flow velocity in a packed configuration. Similarly, the adsorption performance of highly selective affinity systems is shown to be not significantly diminished in the presence of cells.

EXPERIMENTAL

Affinity adsorption systems

In order to obtain a clear understanding of the performance of expanded beds with and without the presence of cells a model affinity adsorption system was chosen in order to eliminate additional complications arising if a system of genuine industrial interest were chosen. The affinity system chosen involves the adsorption of human immunoglobulin G (hIgG) to Protein A Sepharose Fast Flow (Pharmacia LKB Biotechnology, Uppsala, Sweden). Experiments to ascertain the effect of the presence of cells were carried out with commercial preparations of *Saccharomyces cerevisiae* (bakers' yeast). Suspensions of washed cells in 0.1 *M* Tris–HCl buffer pH 7.0 were used in these experiments, as opposed to

using untreated fermentation broth containing cells, in order to be certain that any effects noticed were a direct and sole consequence of the presence of cells rather than being caused by the different physical properties of the broth or the presence of other unspecified compounds in the broth.

In order to demonstrate the benefits of using a highly selective system to minimise interference by cells on adsorption performance, similar experiments were performed with an ion-exchange adsorption system involving the adsorption of bovine serum albumin (BSA) (Sigma, Poole, UK) to Q-Sepharose Fast Flow (Pharmacia LKB Biotechnology).

Finally, we wished to demonstrate the potential of the use of expanded bed adsorption in a more realistic system. The system chosen involved direct adsorption of phosphofructokinase (PFK) from homogenised preparations of *S. cerevisiae*. Such a system was selected to demonstrate that adsorption in expanded beds can be performed in the presence of cell debris in addition to the presence of whole cells. This ability may be of particular interest for large scale industrial implementation as a result of the greater difficult of removing cell debris by centrifugation or filtration.

Characterisation of physical properties of adsorbent beads

Samples of each of the adsorbents used were analysed using an Optomax particle size analyzer, in order to measure the mean bead diameters. The adsorbents were first dyed with Brilliant Blue R dye (Sigma) and the analyser was linked up to a Leitz Laborlux K microscope so that individual beads could be viewed easily to ensure an accurate assessment of their diameters. The diameters of between 400 and 500 beads were analysed for each adsorbent. The measured particle diameters were loaded into a computer program and the mean diameter on a volume basis was calculated. The results are shown in Table I. The densities of the adsorbent beads in buffer were assessed by weighing a 50% (v/v) slurry and using a knowledge of the voidage of the settled phase to calculate the volume, mass and hence the density of the adsorbent beads. The values obtained are also shown in Table I.

Measurement of adsorption isotherms in the presence of cells

A range of concentrations of protein (BSA for adsorption to the strong anion exchanger Q-Sepharose Fast Flow, and hIgG for adsorption to Pro-

TABLE I

PHYSICAL PROPERTIES AND RICHARDSON–ZAKI PARAMETERS FOR VARIOUS EXPERIMENTAL SYSTEMS

The table gives the physical properties of the adsorbents and fluidizing solutions used in experiments to study variation in the height of the expanded bed as a function of liquid flow through the bed. The experimental values of the parameters u_t and n are obtained by fits of the data to eqn. 3. Values of u_t were also calculated from eqn. 1.

Adsorbent type	Adsorbent bead size (μm)	Adsorbent bead density (kg/m^3)	Fluidizing solution	Solution density (kg/m^3)	Solution viscosity (mPas)	u_t (exp.) (mm/s)	u_t (Stokes) (mm/s)	Richardson–Zaki exponent (n)
Q-Sepharose Fast Flow	93.5	1131	0.01 *M* Tris–HCl buffer	1000	1.00	0.7	0.67	4.9
Protein A Sepharose Fast Flow	90	1100	0.1 *M* Tris–HCl buffer	1000	1.005	0.4	0.44	5.2
			5 g/l *S. cerevisiae*	1001	1.05	0.3	0.37	4.9
Cibacron Blue	75	1080	50 m*M* phosphate buffer	1000	1.00	0.2	0.24	4.7
Sepharose Fast Flow	90 elutriated	1090	Yeast cell homogenate	1009	4.02	0.11	0.09	3.9

tein A Sepharose Fast Flow), from 0.5 to 10 mg/ml were made up in separate flasks in 20 ml of Tris–HCl buffer (0.01 *M*, pH 7.0 for the ion-exchange system, and 0.1 *M*, pH 7.5 for the affinity system). A number of sets of flasks were made up in this way, each set of flasks containing a different concentration of cells of *S. cerevisiae*, ranging from 0 to 50 mg dry mass/ml. The adsorbent, in a 1:1 buffer slurry (1 ml in each test), was added to each flask. The flasks were then sealed, to prevent evaporation, and incubated with agitation in a 25°C water bath for between 50 and 70 h to allow equilibrium to occur between the solid and liquid phases. After this period had elapsed, a 1.5-ml sample was removed from each flask into an Eppendorf centrifuge tube, and centrifuged to pellet adsorbent and cells. Controls were performed without added adsorbent, and with and without cells being present. The optical density of each supernatant was measured at 280 nm and the reading was used to obtain the equilibrium concentration of protein in the liquid phase. Measurement of the optical density of the controls confirmed that there was, under these conditions, negligible release from the cells of material that absorbs light at 280 nm. The amount of protein adsorbed to the adsorbent was calculated by mass balance.

Rates of mass transfer to the adsorbents

The rates of protein uptake in the presence of cells were measured using a series of agitated 100-ml beakers. A number of sets of beakers were set up, each with a different concentration of cells of *S. cerevisiae* ranging from 5–50 mg/ml. For the experiments with the ion exchanger Q-Sepharose Fast Flow, 90 ml of solutions of 2.5 mg/ml of BSA in 0.01 *M* Tris–HCl buffer, pH 7.0 were used and subsequently 2 ml of a 1:1 (v/v) adsorbent–Tris–HCl buffer slurry was injected into each beaker. In tests on the affinity system, a solution of 1.0 mg/ml hIgG in 0.1 *M* Tris–HCl buffer, pH 7.5 was used and 1 ml of a 1:1 adsorbent–Tris–HCl buffer slurry was injected into each beaker. These beakers were agitated in a shaking water bath at 25°C to keep the adsorbent and cells in suspension. The experiment was started by injecting the appropriate volume of adsorbent slurry into each beaker. A 1.5-ml sample of the suspension in each beaker was immediately removed and centrifuged to pellet cells and adsorbent particles. The optical density of the supernatant was measured at 280 nm, and the concentration of the adsorbate in solution was calculated by reference to prior calibration of the spectrophotometer. After each subsequent interval of 5 min, a further 1.5-ml sample of the suspension was removed and the process repeated, until the optical density of the supernatant liquors had levelled off to a steady value.

Packed and expanded bed procedures

Two types of column were used in this study. In both cases it was necessary to use a flow distribution system at the base of the column to ensure even distribution of flow over the cross-section of the bed. It was also necessary to fit the distributor with a net or scintered disc of a porosity large enough to allow free passage of particulate material in the inlet stream whilst still retaining adsorbent beads in the column when liquid flow was stopped. A small glass column of 2 cm diameter was used for experiments with Protein A Sepharose Fast Flow in order to reduce the need to use large volumes of this adsorbent. The distributor of this column consisted of a 5-mm glass inlet tube leading into a hemispherical flow distribution volume prior to P160 sintered glass disc (BDH Laboratory Apparatus) with a nominal pore diameter of 160 μm. The 5.0-cm diameter column used for experiments with Cibacron Blue Sepharose Fast Flow was a water-jacketed XK50 column (Pharmacia LKB Biotechnology) fitted with a modified bottom adapter. The 2-cm and 5-cm columns were plumbed with tubing with internal diameters of 1 mm and 2 mm, respectively. Care was taken to prevent any air bubbles from collecting behind the distributor systems as their presence was a common source of bed instability.

The adsorption of pure hIgG onto Protein A Sepharose Fast Flow was tested in packed and expanded bed configurations to examine and compare the efficiencies of the adsorption performance of the adsorbents under these conditions. In experiments in the absence of cells, the concentration of hIgG in the outlet stream from the bed was followed by measuring optical adsorption at 280 nm, and this was used to measure the breakthrough curve. The layout of the apparatus used in these tests is described in full elsewhere [10,12,13]. Two P-6000 (Pharmacia LKB Biotechnology) pumps were used

in the system and could be controlled, either manually or automatically, using a personal computer interfaced to the system via an LCC 500 CI process controller (Pharmacia LKB Biotechnology). One of these pumps was used to pump buffer during equilibration and washing, and eluent during the elution stage, and the second pump was used to pump the adsorbate solution. The adsorbate solutions consisted of 1.0 mg/ml hIgG in 0.1 *M* Tris–HCl buffer, pH 7.5. The UV absorbance of the column outlet stream was measured at 280 nm with a UV-1 monitor (Pharmacia LKB Biotechnology). These signals were recorded on a chart recorder as well as being logged by an FPLC Manager software package (Pharmacia LKB Biotechnology).

Adsorbent slurry was washed in a filter with distilled water to remove the preservative liquor. The washed adsorbent was then poured into the bed, until a suitable settled bed height was attained. The adsorbent was washed *in situ* initially with 0.1 *M* Tris–HCl buffer, and fines were elutriated by washing the bed with buffer at a linear flow velocity 1.5 times that which would be used subsequently for obtaining a stable expanded bed. The flow-rate was then readjusted and washing with buffer continued until the bed had expanded to the required height and the top of the expanded bed remained at a constant level. When the bed was operated in the expanded configuration, the top adapter was lowered to just above the surface of the expanded bed, to avoid the presence of a column of liquid above the bed. During operation in a packed configuration, the top plunger was fitted with the standard net and lowered onto the surface of the bed. The bed was then ready for the application of adsorbate solution. Adsorbate was applied to the bed until the optical density of the liquid leaving the bed had approached that of the bed inlet stream. It was observed that at low levels of bed expansion, the height of the expanded bed dropped slightly during protein adsorption as a result of protein binding to the adsorbent. The flow-rate through the bed was therefore increased gradually in order to maintain a constant extent of bed expansion. Such increases were not found to be necessary when greater degrees of bed expansion were used.

When measurement of the breakthrough curve was complete, the system was switched to buffer delivery, to wash excess adsorbate solution from the column, until the UV absorbance of the column outlet stream had decreased and leveled off. At this stage, the expanded bed was returned to a packed configuration by stopping flow through the bed and allowing the adsorbent to settle. This was followed by lowering the adapter onto the surface of the packed bed. This procedure was not necessary when the adsorption stage had been carried out in a packed bed configuration. Eluent was passed through the bed to remove adsorbed protein from the adsorbent. In general, a large peak of eluted protein was observed and elution was continued until the optical density had reached a steady, low value. The system was again switched to buffer delivery, and buffer was pumped through the column to remove excess eluent from the column. Re-equilibration continued until the pH of the outlet stream had increased back to the original value at the beginning of the run.

For expanded bed adsorption experiments in the presence of cells, a modified procedure had to be adopted during the adsorption as the presence of particulates prevented the direct monitoring of the breakthrough curve of adsorbate by simple on-line measurement of the optical density at 280 nm. Adsorbate solution was pumped through the bed until an amount of adsorbate equal to the theoretical maximum adsorption capacity of the bed (as determined from the results of the batch isotherms) had been applied to the bed. The liquid leaving the expanded bed was collected in 10-ml fraction collector test tubes using a FRAC 300 fraction collector (Pharmacia LKB Biotechnology). The samples were centrifuged to pellet the cells and the optical density of each clarified supernatant was measured at 280 nm. Control experiments confirmed that the measured optical density was an accurate measure of the level of adsorbate in these samples.

Measurements of bed expansion

The characteristics of bed expansion were measured using the apparatus described above for expanded bed adsorption experiments. Liquid flow through the bed was initially increased in discrete steps, until the top surface of the expanded bed appeared to become unstable and diffuse. Then the flow-rate was decreased in discrete steps back to zero. A 15–20 min interval was allowed after each alteration in flow rate to allow the level of the bed to

reach a constant value. Bed height was measured as a function of the liquid superficial linear velocity in the bed. Theoretical values of the terminal velocities of the adsorbent beads were also calculated from Stokes' equation:

$$u_t = \frac{d_p^2(\rho_s - \rho_f)\, g}{18\, \mu} \quad (1)$$

Disruption of cells of S. cerevisiae

Before experiments could be carried out to determine the potential for the use of expanded bed adsorption from cell homogenates, it was necessary to prepare a suitable preparation of disrupted cells. The following protocol was used to disrupt cells of *S. cerevisiae* in order to obtain release of the intracellular enzyme phosphofructokinase. 10 l of a 50 mg/ml suspension of fresh cells of *S. cerevisiae* (Barker's Bakery, Impington, Cambridge) in cold 0.05 *M* phosphate buffer pH 7.2 with 0.5 m*M* EDTA were homogenized in an APV Gaulin continuous Manton press. Before the first pass, the yeast suspension was cooled in ice to a temperature of approximately 4°C. The press was started and whilst the yeast suspension was being fed into the press at the start of the pass, the pressure was raised rapidly to 750 bar. After each pass, the pressure was reduced to atmospheric pressure, and the suspension cooled again in ice to reduce the temperature from about 30–40°C to the starting temperature of 4°C. When this had been achieved, the suspension was fed through the press in another pass and cooled again. This process was repeated for 7 passes. After 7 passes through the Manton press, a sample of the suspension was viewed under an optical microscope, and from a qualitative analysis, it appeared that approximately 80% of the cells had been disrupted. Homogenisation was stopped at this stage, and 2-mercaptoethanol and phenylmethylsulphonyl fluoride (PMSF) were added to the suspension, to concentrations of 5 m*M* and 0.5 m*M*, respectively. The homogenate was refrigerated at 4°C immediately after the disruption process, and was used in tests of expanded bed adsorption over the following three days. No apparent deterioration of the homogenate appeared to occur during this period. Subsequent analysis using an Optomax particle size analyser revealed that between 80–90% of the cells had been disrupted, when compared with a sample of similar size of the whole cell broth with a cell count of about 300 cells. The error was ±5% in the count. The total protein content, of the homogenate was measured to be 2.06 mg/ml. The phosphofructokinase activity of the homongenate was determined by enzyme assay to be 0.183 U/ml.

Purification of phosphofructokinase from yeast cell homogenate

The column used for the purification of phosphofructokinase from a yeast cell homogenate was an XK50 column (Pharmacia LKB Biotechnology) fitted with a modified bottom adapter. The equilibration and washing buffer used in this section was 0.05 *M* phosphate buffer at pH 7.2, containing 0.5 m*M* EDTA. Cibacron Blue Sepharose Fast Flow adsorbent was filtered under vacuum to remove the preservative liquor, and washed with distiled water. Adsorbent was poured into the column and once the bed had settled, buffer was pumped through the system at a superficial flow-rate of 0.76 cm/min, to remove fines and hence reduce the range of sizes of adsorbent beads. The top plunger in the column was lowered to just above the surface of the bed, to prevent a column of liquid forming above the bed surface.

Purification experiments were carried out with a settled bed height of 7 cm which expanded to a height of 25 cm at a flow velocity of 0.5 cm/min (10 ml/min) with phosphate buffer as the irrigating liquid. When a stable, expanded bed had been set up in phosphate buffer, the yeast cell homogenate was applied to the column. As the homogenate was more dense and viscous than buffer, the flow-rate was reduced to 0.31 cm/min (6 ml/min) to maintain the same extent of bed expansion, and to prevent loss of adsorbent from the expanded bed. During the adsorption phase, the bed height was observed to drop slightly as a result of protein binding to the adsorbent, therefore the flow-rate had to be increased at intervals. The top surface of the expanded blue bed could be observed reasonably clearly through the cream coloured homogenate background so that efficient control of flow-rate to achieve the required degree of bed expansion was possible.

At the end of the adsorption phase, wash solution was passed through the bed at a flow-rate of 0.5 cm/min (10 ml/min) until particulate material had been

washed from the bed, and until the optical density of the column outlet stream had decreased to a low value. Adsorbed proteins were eluted from the adsorbent in a packed configuration to reduce dilution of the product in the eluate. The eluent (1 *M* NaCl in water) delivery rate was 1 cm/min and samples of the eluate were taken at regular intervals. Once the optical density in the column outlet stream had decreased to zero, phosphate buffer was again passed through the bed to re-equilibrate the adsorbent.

The optical density at 280 nm of the stream leaving the bed was measured continuously with a UV-1 monitor (Pharmacia LKB Biotechnology) and recorded on a chart recorder. Samples of the flow-through were collected at regular intervals throughout the experiment and stored on ice. Each sample was assayed for phosphofructokinase activity and total protein content using the methods described below.

Assay of phosphofructokinase

Phosphofructokinase (ATP: D-fructose-6-phosphate-1-phosphotransferase; EC 2.7.1.11) was assayed in a coupled system in which the phosphorylation of fructose 6-phosphate to form fructose 1,6-biphosphate was indicated by the NADH dependent reduction of dihydroxyacetone phosphate to glycerol 3-phosphate [16]. Aldolase (EC 4.1.2.13), triosephosphate isomerase (EC 5.3.1.1) and glycerol-3-phosphate dehydrogenase were used in the assay as auxiliary enzymes. One unit of enzyme activity was defined as the amount producing 1 μmol of fructose 1,6-biphosphate per minute under the assay conditions. Enzyme eluted from Cibacron Blue Sepharose Fast Flow was desalted before assay by molecular exclusion chromatography on a Pharmacia PD-10 column with 0.05 *M* Tris–HCl buffer, pH 7.2 as running buffer.

Measurement of protein content

The protein content of samples obtained during the purification of phosphofructokinase from a yeast cell homogenate was determined by the Bradford assay using Coomassie Protein Assay Reagent (Pierce) [17].

Gel electrophoresis

Samples were prepared for SDS gel electrophoresis by filtering 100 μl of the sample through a Nalgene 0.2 μm filter to remove particulate material and desalting the filtrate using a PD-10 column (Pharmacia LKB Biotechnology). Then 10 μl of 2-mercaptoethanol, 3 μl of bromophenol blue solution (to detect the migrating front), a few granules of Na_2HPO_4 (to increase the pH), and 2 mg of sodium dodecylsulphate were added. The solution was boiled in water for 10 min and after cooling 1 μl samples were applied to a 25% polyacrylamide gel which was subjected to electrophoresis on a Phast-System (Pharmacia LKB Biotechnology) at a constant current of 100 mA for 75 VhA.

RESULTS

Effect of cells on adsorption characteristics

In order that an affinity adsorbent can be used in direct broth extraction techniques it is important to ascertain that the adsorption properties are not seriously impaired in the presence of cells. Experiments were carried out to determine the effect of cells on equilibrium and mass transfer characteristics of the two adsorption systems used in this study.

(i) Equilibrium adsorption isotherms. The effects of the presence of cells of *S. cerevisiae* on the equilibrium characteristics of the adsorption of pure hIgG to the highly selective affinity adsorbent Protein A Fast Flow were determined by the measurement of adsorption isotherms. Fig. 2b shows the results obtained which indicate comparatively little alteration of the shape of the isotherms even at concentrations of cells as high as 30 mg dry mass/ml. Similar experiments were carried out with a much less selective adsorption system namely the adsorption of BSA to Q-Sepharose Fast Flow (Fig. 2a) and the results indicate a far greater reduction in the adsorption capacity in the presence of cells.

A more quantitative assessment of the results was performed by fitting the data to the Langmuir isotherm. In all cases, a good quantitative agreement was shown to an equation of the form [18]

$$q^* = \frac{q_m c^*}{(K_d + c^*)} \qquad (2)$$

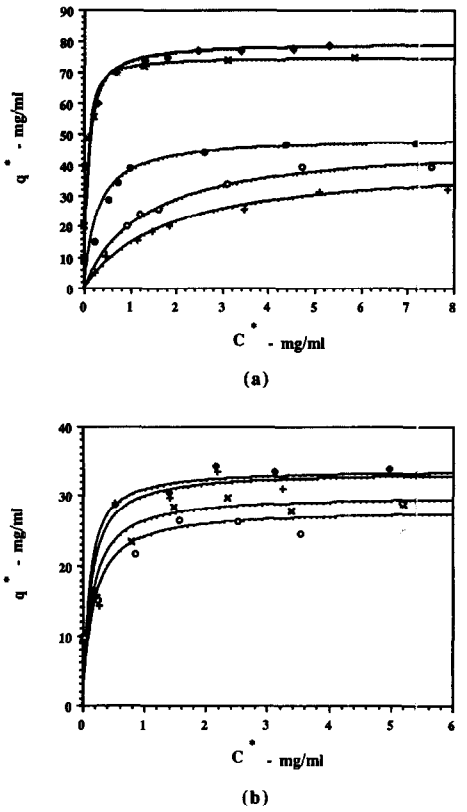

Fig. 2. Isotherms for the adsorption of proteins in the presence of cells. Adsorption isotherms were measured as described in the text for the adsorption of proteins to the adsorbents in the presence of various concentrations of cells of *S. cerevisiae*. (a) The adsorption of bovine serum albumin to Q-Sepharose Fast Flow in 0.01 *M* Tris–HCl buffer, pH 7.0 at 25°C. The concentration of cells (mg dry mass/ml) present in each experiment were: 0 (◆); 5 (×); 10 (●); 30 (○); 50 (+). (b) The adsorption of hIgG to Protein A Sepharose CL4B in 0.1 *M* Tris–HCl buffer, pH 7.5 at 25°C. The concentration of cells (mg/ml) present in each experiment were: 0 (◆); 5 (+); 15 (×); 30 (○). In both cases, the solid line shows the best fit to a Langmuir type of isotherm of the form described in eqn. 2.

where q_m and K_d are the maximum capacity and the dissociation constant of the adsorption interaction. Although adsorption of protein was occurring in the presence of simultaneous adsorption of cells to the adsorbent, the isotherms for protein adsorption continued to be adequately described by a Langmuir type adsorption isotherm. Such behaviour might not be expected in the presence of multi-component adsorption, but can readily be explained as a consequence of the very small degree of adsorption of the total amount of cells present that become bound to the adsorbent in these experiments. Hence the concentration of cells in the liquid phase at equilibrium remains constant for all points on the protein adsorption isotherm and thus the shape of this isotherm remains hyperbolic although the amount of protein adsorbed is reduced. The effect of the presence of cells on the values of q_m and K_d are shown in Table II. The values given in Table II demonstrate that at a cell concentration of 30 mg dry mass/ml, the maximum adsorption capacity of the affinity system is reduced by 17% with a 68% increase in the dissociation constant. In the presence of the same concentration of cells, the equivalent values for the ion-exchange system are altered more drastically, with a 49% decrease in the maximum capacity and an increase in the dissociation constant by almost two orders of magnitude. Hence the available capacity for protein adsorption will be much more significantly reduced in the presence of cells for the ion-exchange system than the affinity system. A similar reduction of the capacity of Q-Sepharose Fast Flow for BSA has also been observed in the presence of cells of *Alcaligenes eutrophus* [13].

TABLE II

ADSORPTION EQUILIBRIUM CHARACTERISTICS IN THE PRESENCE OF CELLS

Adsorption isotherms were measured in the presence of various concentrations of cells of *S. cerevisiae* as described in the text and shown in Fig. 2. The data obtained was fitted to Langmuir adsorption isotherms of the form of eqn. 2 and the parameters for the maximum capacity of the adsorbent, q_m, and the dissociation constant of the adsorbent–protein complex (K_d) determined.

Adsorbent	Adsorbate	*S. cerevisiae* concentration (mg/ml)	q_m (mg/ml)	K_d (mg/ml)
Q-Sepharose Fast Flow	BSA	0	80	0.02
		5	75.2	0.05
		10	49.0	0.28
		30	47.6	1.31
		50	41.1	1.80
Protein A Sepharose Fast Flow	hIgG	0	33.8	0.095
		5	33.5	0.12
		15	30.1	0.14
		30	28.0	0.16

(ii) Mass transfer to the adsorbent. The experiments to measure the equilibrium characteristics of adsorption were performed under conditions where sufficient time was allowed for equilibrium to be attained between the liquid and solid phases. Whereas the equilibrium characteristics give knowledge of the maximum amount of adsorbate that can be adsorbed when a bed is contacted with a stream containing adsorbate at a particular concentration, it is also very important to have knowledge on the rate at which mass is transfered between phases as this parameter will have a crucial effect on the capacity of the adsorbent that can be used efficiently under the given operating conditions. Hence a check was made to determine that the rates of mass transfer to the affinity adsorbent were not greatly diminished in the presence of cells. The rates of mass transfer were studied in batch uptake experiments in agitated flasks. Some typical uptake curves for the adsorption of hIgG onto Protein A Sepharose Fast Flow are shown in Fig. 3b. For comparison, similar experiments were again also carried out with the less selective system involving the adsorption of BSA to Q-Sepharose Fast Flow (Fig. 3a). The results show qualitatively that the presence of cells has less effect on the affinity system than the ion-exchange system. A method for the determination of mass transfer parameters such as a film mass transfer coefficient and an effective diffusivity for diffusion of adsorbate within the porous particle has been described previously [13,19,20]. The application of this method to the results presented here yields the values of the parameters shown in Table III. It can be seen that the presence of cells of *S. cerevisiae* has very little effect on either the value of the film mass transfer coefficient nor the effective diffusivity pertaining in the affinity adsorption system. However, the values of the film mass transfer coefficient are significantly reduced in the ion-exchange system. The results show agreement with similar experiments carried out to determine the effect of the presence of cells of *Alcaligenes eutrophus* on the same parameters although those studies indicated an even greater reduction in the value of the effective diffusivity within the ion-exchange particle in the presence of cells [13].

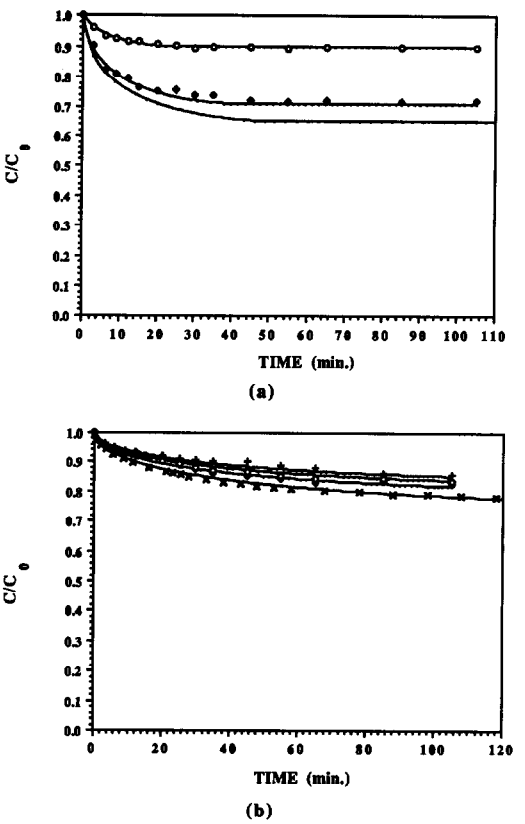

Fig. 3. Batch uptake profiles for the adsorption of proteins in the presence of cells. The rates of mass transfer of protein to the adsorbents were studied in batch uptake experiments as described in the text in the presence of various concentrations of the cells of *S. cerevisiae*. (a) The adsorption of bovine serum albumin to Q-Sepharose Fast Flow in 0.01 *M* Tris–HCl buffer, pH 7.0. The concentration of cells (mg dry mass/ml) present in each experiment were: 0 (×); 5 (◆); 50 (○). (b) The adsorption of hIgG to Protein A Sepharose CL4B in 0.1 *M* Tris–HCl buffer, pH 7.5. The concentration of cells (mg/ml) present in each experiment were: 0 (×); 5 (◆); 15 (○); 30 (+). In both sets of experiments, the solid lines represent the best fit theoretical simulation that achieve the best fit to the data on the assumption that film and pore diffusion govern mass transfer to the adsorbent bead.

Characterisation of the hydrodynamic behaviour of stable, expanded beds

The expansion characteristics of a bed of Protein A Sepharose Fast Flow as a function of the velocity of flow through the bed are shown in Fig. 4, both in the presence and absence of cells. The bed was found to expand to a greater extent at a particular velocity of flow in the presence of cells. Similar results have been found in studies on the Q-Sepharose Fast

TABLE III

MASS TRANSFER PARAMETERS MEASURED IN THE PRESENCE OF CELLS

Mass transfer parameters were measured by analysis of batch adsorption experiments as described in the text. Results of the form shown in Fig. 3 were analysed by computer methods described elsewhere to determine values of the mass transfer coefficient k_f and the effective diffusivity of adsorbate within the porous adsorbent bead (D_e). The values of the free solution diffusivities estimated from correlations as described previously are also given for comparison. All data refer to a temperature of 20°C.

Adsorbent	Adsorbate	D_{AB} (10^{-11} m^2/s)	*S. cerevisiae* concentration (mg/ml)	D_e (10^{-11} m^2/s)	k_f (10^{-6} m/s)
Q-Sepharose Fast Flow	BSA	7.4	0	1.0	7.7
			5	0.9	5.0
			50	0.85	1.0
Protein A Sepharose Fast Flow	hIgG	5.6	0	0.25	6.3
			5	0.25	6.0
			15	0.22	5.2
			30	0.20	4.5

Flow. In all cases, the results were found to fit well to a Richardson–Zaki equation [21] of the form:

$$u = u_t \varepsilon^n \quad (3)$$

The values determined for the Richardson–Zaki parameter (n) and the terminal velocity u_t are shown in Table I. The values of the Richardson–Zaki parameter (n) are close to value of 4.8 normally used in the laminar flow regime [22–24] although the value determined in a yeast cell homogenate differs somewhat from this value. The experimentally determined values of u_t agree quite well with the values predicted from the Stokes' equation on substitution of appropriate values for the physical characteristics of the adsorbent beads and the fluidizing liquid. The observed decrease in the terminal velocity, u_t, of the adsorbent beads in the presence of cells is to be expected from the higher apparent density and viscosity of the cell suspensions than that of pure Tris buffer.

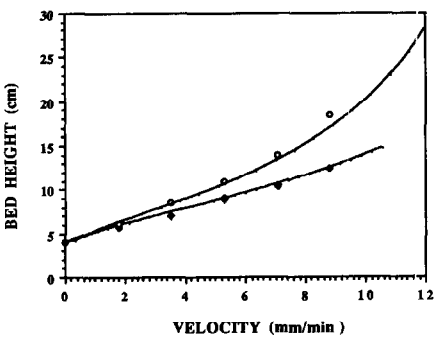

Fig. 4. Bed expansion characteristics of a bed of Protein A Sepharose Fast Flow. The characteristics of expansion of a bed of Protein A Sepharose Fast Flow were determined in a 2-cm column at 20°C. The height of the settled bed of adsorbent was 4 cm. Bed expansion was performed in the presence of the following solutions: 0.1 *M* Tris–HCl buffer, pH 7.5 (◆); 5.0 mg ml^{-1} *S. cerevisiae* cells in 0.1 *M* Tris–HCl buffer, pH 7.5 (○).

Residence time distributions were determined to establish the pattern of liquid flow through the expanded bed of Protein A Sepharose Fast Flow. This was achieved by measuring the variation in the concentration of tracer in the outlet stream from the bed following the injection of a sharp pulse of tracer (0.5% acetone) in the bed inlet. The resultant curves were examined to determine whether liquid flow through the bed was nearer that of plug flow or whether the expanded bed system behaved more like a well mixed system in a manner similar to that used with identical studies of expanded beds of ion exchange adsorbent [11,12]. The values for Pe obtained were less than 0.01 and this is taken as evidence that liquid flow through the bed is close to plug flow [25]. Since liquid flow was observed to be close to plug flow, this was in turn taken as being evidence that there was probably very little circulation and mixing of the adsorbent beads within the bed. This suggestion was tested by making visual observations on the mixing of a small sample of dyed

adsorbent beads added to the top of the bed and these tests confirmed very little apparent mixing.

It appears from the hydrodynamic studies that the expanded bed exhibits very little mixing of the adsorbent beads and that liquid flow through the bed approximates the characteristics of plug flow. Both these observations suggest that the expanded bed might show adsorption behaviour similar to that observed in a packed bed. The validity of this suggestion is confirmed in the next section.

Comparison of adsorption breakthrough profiles in packed and expanded beds

A comparison was made between the efficiency of protein adsorption in a fixed and expanded bed, by examining the adsorption of hIgG to Protein A Sepharose Fast Flow in a 2.0-cm diameter column (Fig. 5). In these experiments, adsorbate was applied to the same amount of adsorbent at the same volumetric flow-rate with the bed in either a packed or expanded configuration. It is apparent that when the adsorbent bed is expanded to approximately 3–4 times its settled bed height, there is little or no difference between the efficiency of protein adsorption in the fixed or expanded bed modes. The breakthrough curves obtained for both modes of operation are virtually identical. It is important to expand the bed gradually and to allow the bed to stabilize before beginning the protein adsorption phase. Hence the expanded bed appears to behave like a packed bed except that its interstitial voidage is greater. This has led us to suggest that models that have been developed to predict the performance of the adsorption phase in fixed bed systems can also be used to predict expanded bed adsorption performance provided that the greater voidage of the expanded bed is taken into account [10–13].

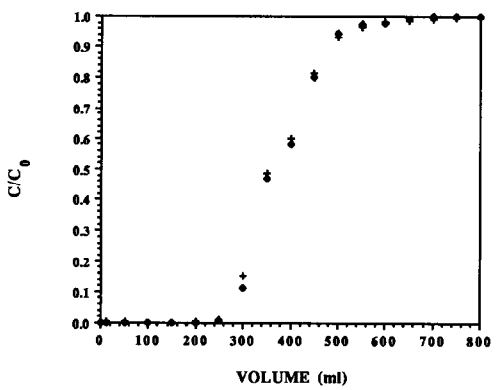

Fig. 5. Packed and expanded bed adsorption of hIgG onto Protein A Sepharose Fast Flow. Breakthrough curves for the adsorption of a solution of 1.0 mg/ml hIgG in 0.1 *M* Tris–HCl buffer, pH 7.5 to Protein A Sepharose Fast Flow in a 2.0-cm column were measured with the bed in either an expanded configuration (+) (expanded bed height *ca.* 12 cm) or a packed configuration (◆) (packed bed height = 4 cm). Both experiments were carried out at a linear flow velocity of 0.95 cm/min (3 ml/min).

Expanded bed adsorption of immunoglobulin G in the presence of intact cells of S. cerevisiae

As indicated by the results of the equilibrium and mass transfer batch uptake experiments described above, the presence of intact cells of *S. cerevisiae* has little effect on the adsorption of hIgG to Protein A

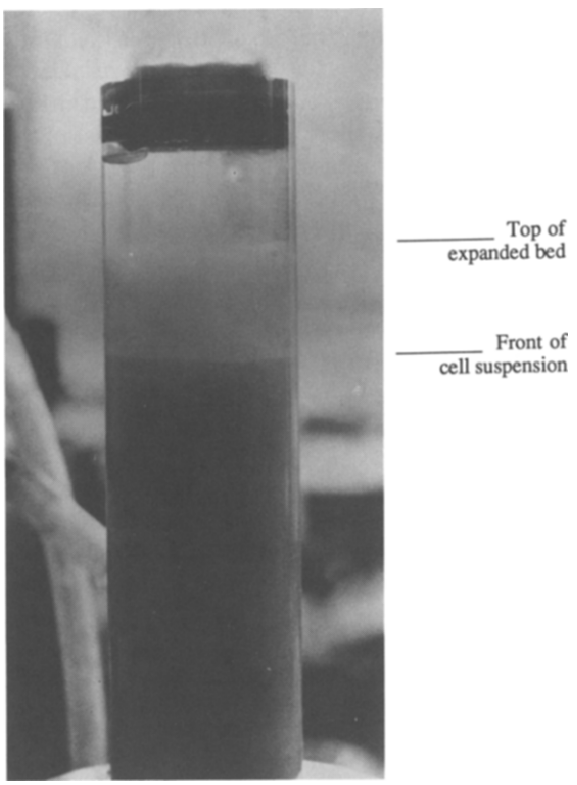

Fig. 6. Passage of a cell suspension through a stable, expanded bed. The photograph shows a front of a suspension of yeast cells passing through an expanded bed of a Sepharose Fast Flow adsorbent. The top adapter has been lifted up from its usual position which would normally be closer to the top of the bed. The top of the expanded bed can be seen to be stable and the front of the cells is very sharp indicating very little mixing of the liquid within the bed.

Sepharose Fast Flow. Hence experiments were carried out to confirm that the shape of the breakthrough for adsorption of IgG to expanded beds of Protein A Sepharose Fast Flow was similarly unaffected by the presence of cells. The passage of the front of the yeast cell suspension is shown in Fig. 6. In this demonstration, the top adapter has been raised from its normal position so that the stable, non-diffuse top of the expanded bed can be clearly seen. Further down the bed, the front of yeast cells moving freely through the bed can be observed to be sharp, with no evidence of gross mixing of the liquid phase. These visual observations confirm the stability of the expanded bed deduced from the other experiments described above.

Measurement of breakthrough curves for expanded bed adsorption in the presence of varying concentrations *S. cerevisiae* in a 2.0-cm diameter column, showed that the adsorption performance appeared to be unaffected by the presence of cells (Fig. 7). The affinity adsorbent adsorbed hIgG efficiently, although there was a small decrease in the efficiency of adsorption in the latter stages of the breakthrough curve as evidenced by a reduction in the gradient of the curves when a value of C/C_0 of 0.8 was exceeded. This effect was more pronounced

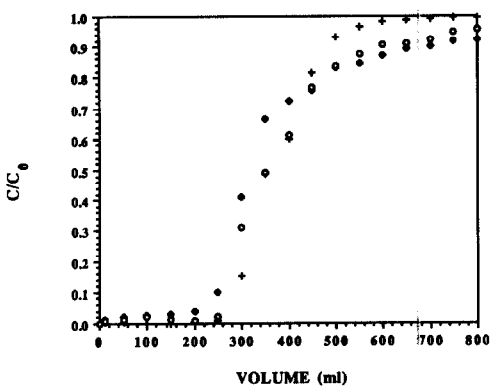

Fig. 7. Effect of the presence of yeast cells on expanded bed adsorption of hIgG onto Protein A Sepharose Fast Flow. Breakthrough curves were measured for the adsorption of a 1 mg/ml solution of hIgG in various liquids onto an expanded bed of Protein A Sepharose in a 2-cm diameter column. The height of the settled bed of adsorbent was 4 cm and an appropriate flow-rate was used to expand the bed to a height of 12 cm during measurement of the breakthrough curves. The solutions used were: 0.1 *M* Tris–HCl buffer, pH 7.5 (+); 0.1 *M* Tris–HCl buffer, pH 7.5 containing 5 mg/ml cells (○); 0.1 *M* Tris–HCl buffer, pH 7.5 containing 15 mg/ml cells (◆).

at higher cell concentrations and is likely to be a direct consequence of the slight reductions in the values of the mass transfer parameters observed. In any case, such a decrease in adsorption performance at these late stages of the breakthrough curve is unlikely to be important in industrial practice as the adsorption phase would be terminated before such high values of C/C_0 where reached in order to prevent loss of adsorbate in the flow through.

No problems were observed associated with the passage of concentrated cell suspensions through the expanded bed. Measurement of the cell concentration in the liquid that had flowed through the bed indicated that the hold-up of cells within the bed was negligible. The observations that adsorption performance of the affinity adsorbent was not significantly affected by the presence of cells indicated that the cells were not adsorbing strongly to the surface of the adsorbent beads. This was confirmed by observation that the adsorbent retained its original white colour, and by the absence of cells bound to the adsorbent surface, which was qualitatively validated from an inspection of a sample of the adsorbent under an optical microscope.

At the end of the adsorption phase, it is necessary to wash particulates and unadsorbed proteins out of the voids of the bed. This stage is also carried out with the bed in the expanded configuration in order to prevent the trapping of particulate material in the bed. Previous experiments had indicated that switching the liquid flow through the bed from a cell suspension to a less viscous and less dense wash buffer could result in destabilisation of the expanded bed with undesirable mixing of the adsorbent beads and, in extreme cases, loss of adsorbent from the bed. However, when cell suspension is followed by a wash solution of greater density and viscosity, the stable front of this solution was found to be very effective in sweeping particulates from the bed without compromising bed stability. These recommendations are illustrated in Fig. 8 which demonstrates that a 25% (v/v) solution of glycerol is far more efficient than a simple Tris buffer at removing cells from the expanded bed.

It appears therefore that affinity adsorbents can be used in the purification of proteins in the presence of cells without loss in the adsorption performance compared to that which would have been achieved if clarified material had been applied to the same

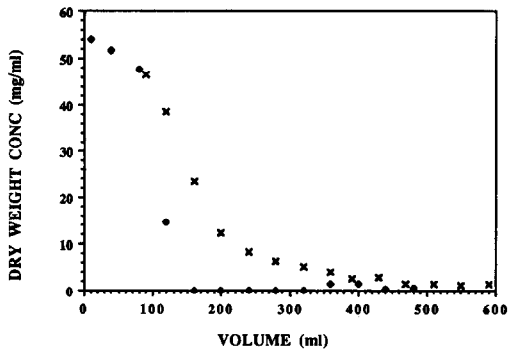

Fig. 8. Removal of yeast cells from an expanded bed during the wash stage. A comparison was made of the efficiencies of two wash buffers for removing cells from an expanded bed of Protein A Sepharose Fast Flow contacted with a suspension of yeast cells of 50 mg dry mass/ml. The buffers used were: (×) 0.1 *M* Tris–HCl buffer, pH 7.5; (◆) 25% (v/v) glycerol in 0.1 *M* Tris–HCl buffer, pH 7.5.

amount of adsorbent in a packed configuration at the same volumetric flow-rate.

Direct purification of phosphofructokinase from yeast cell homogenates

Experiments were carried out to assess the feasibility of using expanded beds of Cibacron Blue Sepharose Fast Flow for the direct purification of phosphofructokinase from yeast cell homogenate. The results of a typical experiment are shown in Fig. 9. In this particular experiment, yeast cell homogenate was applied to the bed until the complete breakthrough curve for PFK had been followed, *i.e.*, the level of PFK in the flow through was equal to that in the material applied to the bed. In the experiment shown in Fig. 9, a steep rise in optical density is observed after 400 ml have been applied to the column which indicates the front of the particulate material in the liquid leaving the bed. This is mirrored by a similar rise in the total soluble protein concentration as proteins not adsorbed by the affinity adsorbent pass through the bed. However, a subsequent more gradual rise in protein concentration is also observed which is assumed to represent the breakthrough of other proteins once the capacity of the adsorbent for each component is exhausted. Complete removal of PFK from the flow through was achieved until just under 1 litre of homogenate had been applied to the bed. The subsequent breakthrough of PFK was sharp and the activity rose to its level in the incoming stream before the start of the wash phase. When PFK has fully broken through the bed, the protein concentration in the exit stream is less than that in the input stream which indicates that other components in the homogenate are continuing to be adsorbed to the adsorbent even though its capacity for PFK is saturated. The adsorbent was not fouled by cell debris during the adsorption phase and the expanded bed did not become unstable in the presence of homogenate. No channels were observed to form as a result of homogenate flowing preferentially through certain parts of the bed and the surface of the bed remained stable throughout the adsorption runs.

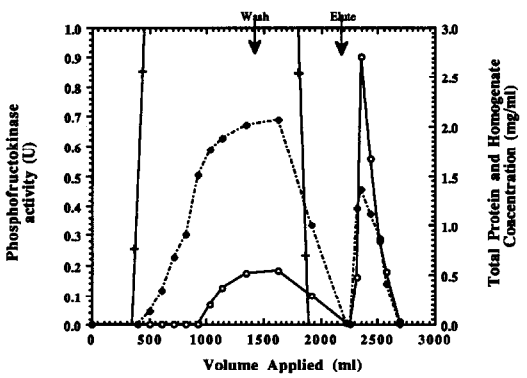

Fig. 9. Purification of phosphofructokinase from yeast cell homogenate on an expanded bed of Cibacron Blue Sepharose Fast Flow. The chromatographic protocol used to purify phosphofructokinase from a feedstock of disrupted yeast cells broken by high pressure homogenisation is described in the text. Samples collected during chromatography were analysed for PFK activity (○), and total protein content (◆). The optical density at 280 nm of the stream leaving the bed was monitored continuously (+) and is a measure of the presence of particulates in this stream. Washing was performed with 10% glycerol in 50 m*M* phosphate buffer and the eluent was 1 *M* NaCl. The various stages of the procedure were performed at different flow-rates as described in the text.

At the end of the adsorption phase, residual particulate material was successfully washed from the expanded bed using 10% (v/v) glycerol in phosphate buffer. Fig. 9 demonstrates that less than 2 expanded bed volumes of the wash solution were required to remove particulate material completely from the bed as evidenced by the rapid decline in optical activity of the outlet stream by this point. Total protein and PFK were also observed to fall to very low values by the end of the wash phase. Following washing, the bed was returned to a packed mode and adsorbed proteins were eluted

from the bed with 1 *M* NaCl. The adsorbed proteins were eluted in a sharp peak with the activity of PFK being very similar to that of total protein. There appeared to be no particulate material in the eluent as judged by its the lack of turbidity and the absence of particles on examination under the microscope. This result indicates the considerable success of the expanded bed protocol in eliminating the need for a specific step to remove particulate matter prior to the adsorption procedure.

The success of the purification of PFK was monitored by analysing the protein content of various collected samples by SDS–polyacrylamide gel electrophoresis (Fig. 10). PFK would be expected to run as two bands with high relative molecular masses of 112 000 and 118 000 [26]. The presence of two bands that may correspond to PFK can be seen in a sample taken at the peak of enzyme activity in the eluent although the relative molecular masses of these bands are in the region of 95 000–100 000, *i.e.*, somewhat smaller than those reported previously. The intensities of these bands verify that PFK is indeed more concentrated in the eluent than in the homogenate as the relative intensity of these bands is much greater than the equivalent bands in the homogenate. Many different proteins are observed to be present in the homogenate. However, the lack of complete selectivity of Cibacron Blue for PFK under these conditions is evidenced by the presence of a number of protein bands other than PFK in the eluate. Further proof of the complete removal of PFK during the adsorption phase is given by the absence of bands corresponding to PFK in the flow through collected during this period.

The purification of PFK can also be assessed by reference to a purification table (Table IV). The table shows that the specific activity of PFK was increased in the eluate by a factor of 5.2. Overall yields of PFK were excellent with 98% of the activity applied in the column being accounted for in the

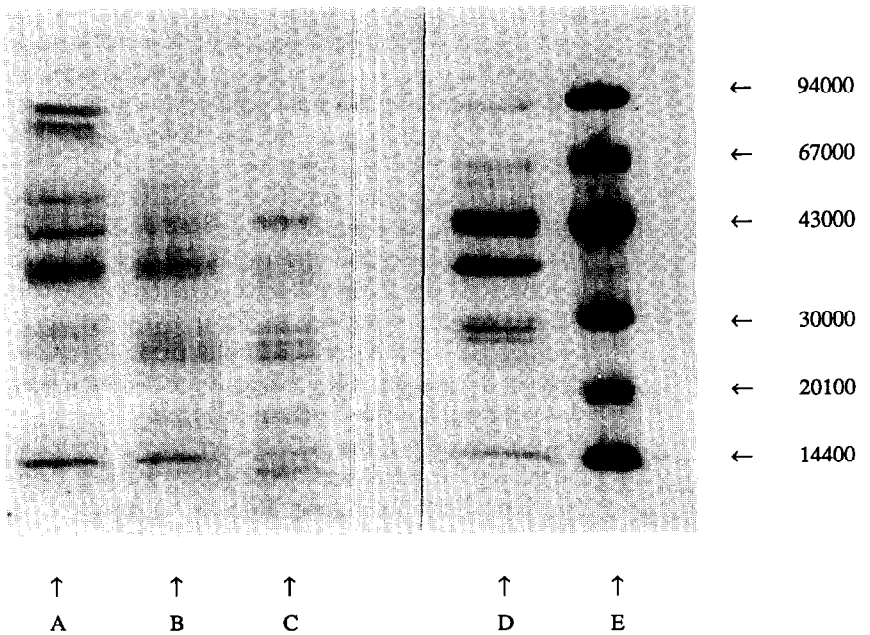

Fig. 10. Analysis of phosphofructokinase purification by SDS gel electrophoresis. Samples from the purification procedure described in Fig. 8 and Table IV were analysed by SDS gel electrophoresis as described in the text. The tracks were as follows: yeast cell homogenate as applied to the bed (track D); flow through during the adsorption stage (track C); eluate (taken at start of total protein peak) (track B); eluate (taken at peak of PFK activity) (track A). The marker proteins for the determination of subunit relative molecular masses (track E) were: phosphorylase *b* (rabbit muscle), 94 000; albumin (bovine serum), 67 000; ovalbumin (egg white), 43 000; carbonic anhydrase (bovine erythrocyte), 30 000; trypsin inhibitor (soybean), 20 100; α-lactalbumin (bovine milk), 14 400.

TABLE IV

PURIFICATION OF PHOSPHOFRUCTOKINASE (PFK) FROM YEAST CELL HOMOGENATE

An experiment was carried out to purify phosphofructokinase from a crude yeast cell homogenate using an expanded bed of Cibacron Blue Sepharose Fast Flow. The experimental protocol was as described in the text and the chromatogram from the run is shown in Fig. 9. Samples collected during chromatography were analysed for phosphofructokinase activity and total protein content and allowed the purification table to be calculated.

Purification step	Volume (ml)	Total PFK activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification factor
Homogenate	1225	225	2523	0.089	1
Flow through	1225	80	1750	0.047	–
Wash	975	53	592	0.089	–
Eluate	700	88	192	0.46	5.2

collected samples. However, the yield of PFK in the eluate was comparatively low (37%) as a direct result of the over prolonged adsorption phase used to measure the full breakthrough curve of PFK. A substantial proportion of the applied PFK appeared in the flow through from the bed and the wash. Other experiments were carried out in which application of homogenate was stopped just before the anticipated start of PFK breakthrough in order to simulate more closely the type of purification protocol that would be carried out in practise which would minimise any loss of PFK in the flow through from the bed. These experiments indicated that it was possible to obtain approximately a 10-fold purification of this enzyme from yeast cell homogenate. The inability to obtain yet higher degrees of purification is a consequence of the lack of specificity of Cibacron Blue Sepharose Fast Flow for this enzyme rather than a consequence of using an expanded bed. Cibacron Blue is reported to adsorb a wide variety of proteins [27] so the adsorption of multiple components would be expected when this adsorbent was contacted with a crude feedstock such as yeast cell homogenate. It might be possible to obtain a higher degree of purification if a protocol for the elution stage involving either a gradient of eluent or a biospecific eluent had been used for the selective removal of adsorbed PFK.

The results of this section have demonstrated that affinity adsorbents can be used in expanded bed protocols for the direct purification of proteins from preparations of disrupted cells.

DISCUSSION

This study of expanded beds of affinity adsorbents in model situations has confirmed the potential of this mode of operation for the direct adsorption of proteins from particulate-containing feedstocks. It has been shown that both whole cells and preparation of disrupted cells can be passed through the expanded bed with remarkably little detrimental effect on the adsorption properties of the bed. Under these circumstances the expanded bed can be considered to be a direct analogy of a packed bed system.

We anticipate that the introduction of this technology will have important impact on certain downstream processing flow sheets as it will eliminate the need to include a step for the removal of particulates before a feedstock can be applied to a bed of adsorbent. However, it is important to attempt to implement it only in such situations to which this operating mode is suited. There will inevitably be many examples of important industrial downstream processing operations where such an approach is not suited. One such example would be in situations where the cells grow as extended chains or as a dense mat as is common in some antibiotic fermentations. It would not be possible to pass such cellular material through an expanded bed without causing gross mixing of the adsorbent beads. Other problems will arise when the liquid being passed through the bed has a very high viscosity as would occur if cells have been disrupted under conditions in which the released nucleic acid polymers are not broken down in length.

The choice of a suitable adsorbent for use in an expanded bed procedure of the type described above needs to be made with care. Both the physical and adsorption properties of the adsorbent need to be considered. With regard to the physical aspects, the adsorbent bead needs to have an appropriate combination of density and bead size such that the required degree of bed expansion can be achieved at a linear flow velocity that is suitable to obtain efficient adsorption of the adsorbate within a reasonable time. The adsorbent beds must have a constrained distribution of particle sizes, sufficient

to achieve a stable expanded bed but not so broad that it is impossible to fluidize the largest beads without elutriation of the smallest ones. The small size of the adsorbent particles used in this study (70–90 μm), coupled with the low density differences between the adsorbents and the liquids being processed results in adsorbents based on the Sepharose Fast Flow matrix being near the lower limits acceptable use in expanded bed protocols. The expansion characteristics of the adsorbent bed are susceptible to small changes in the rheological properties of the liquid passing through it thus complicating control of the expanded height. Similarly, the flow velocities at which it was possible to pass liquid through the expanded beds whilst achieving the required degree of bed expansion were small and might be inconveniently low for some practical applications where it is desirable to load material in a short time to prevent product deterioration. It may not be appropriate to use beads with significantly larger diameters to overcome these problems as the result of the poor kinetics of adsorption that may be a feature of such adsorbents. Hence, it is important to use new adsorbents with densities higher than those in the experiments described here. However, alternative materials must maintain the generally good features shown by adsorbents based on the Sepharose Fast Flow matrix.

Other considerations governing the choice of suitable adsorbents for use in expanded bed procedures are similar to those to which any adsorbent selected for use in direct broth recovery techniques must comply. The porous structure of the bed must be such that it is resistant to fouling and degradation when operated in crude process liquids. The ligand attached to the adsorbent must also be suitable for operation in crude process liquids and ideal ligands are not always available. The choice of ligand is often complicated by the need to compromise between ligand stability and specificity for adsorbing species. The use of robust ligands, such as ion-exchange or hydrophobic interaction groups, often results in low capacities for the desired solute as a result of lack of specificity for this compound. Conversely, the use of ligands with the requisite high specificity is often frustrated by the fragility of such ligands when operated in the harsh operating cycles associated with the processing of crude feedstocks. Such problems are particularly acute when the highly selective ligands are proteins such as antibodies. Whatever ligand is eventually chosen, the resultant adsorbent must have equilibrium and mass transfer characteristics suitable for efficient operation under the flow conditions needed to obtain a stable expanded bed.

The success of expanded bed purification depends crucially on the ability to be able to set up a stable, expanded bed. It is important to assess whether stable beds can be achieved in industrial column with diameters many times greater than those used in this laboratory study. The ratio of the diameter of the bed to the diameter of the adsorbent particles used in these experiments is at least 200, suggesting that bed stability was not a direct result of wall effects in the small column. The ability to be able to achieve an even distribution of flow across the whole surface of the bed has already been achieved in the design of columns intended for use in packed bed chromatography on a large scale. However, it is necessary to make appropriate modifications to ensure that particulates will be able to pass through the flow distribution system. Problems may also arise when processing thick feedstocks associated with observing the top surface of the expanded bed in order to confirm its stability and to determine its position in order to achieve the required degree of bed expansion by altering the bed flow-rate.

We anticipate the introduction of expanded bed purification protocols into a number of industrial scale protein purification flowsheets in the near future.

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ABBREVIATIONS AND SYMBOLS

ATP	adenosine triphosphate
BSA	bovine serum albumin
EDTA	ethylenediaminetetraacetic acid

hIgG	human immunoglobulin G
NADH	nicotinamide adenine dinucleotide
PMSF	phenylmethylsulphonylfluoride
PFK	phosphofructokinase
SDS-PAGE	sodium dodecyl sulphate–polyacrylamide gel electrophoresis
Tris	tris(hydroxymethyl)aminomethane
UV	ultraviolet
C	liquid phase concentration
c^*	equilibrium liquid concentration per unit bed volume
C_0	initial, or inlet concentration of adsorbate
d_p	mean particle diameter
D_{AB}	diffusion coefficient in bulk liquid phase
D_e	effective diffusion coefficient within porous adsorbent bed
g	gravitational constant
k_f	liquid film mass transfer coefficient
K_d	Langmuir isotherm constant (dissociation constant)
M_A	relative molecular mass of substance A
n	Richardson-Zaki coefficient
q	amount of adsorbate bound to adsorbent
q^*	amount of adsorbate bound to adsorbent at equilibrium with liquid phase
q_m	Langmuir isotherm constant (maximum adsorption capacity)
u	superficial liquid velocity
u_t	terminal velocity of adsorbent bead
ε	bed voidage
μ	apparent liquid viscosity
ρ_s	liquid density
ρ_b	particle density

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